POSTGRADUATE MEDICAL SCIENCE

Understanding cancer

From basic science to clinical practice

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and

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Introduction to cancer

1.1 Introductory note

Cancer is a common and widely publicized disease, and in spite of ever increasing efforts to understand it as a process, its incidence in the population is rising. The main reason for this is the close correlation of the number of cancer cases with increasing age of patients, and the number of more aged people, in Western society at least, is rising. It used to be suggested that some aspect of the ageing process increased the susceptibility to cancer, perhaps by impairing immune surveillance, however, it is now generally accepted that the relationship of many cancers to increasing age is rather a reflection of the time required to accumulate a critical number of genetic abnormalities for a cancer to arise. Cancer may affect any organ or tissue, but while some cancers are common, e.g. lung, breast, skin, gut and prostate, others are very rare; those affecting young people often being amongst the rarest.

As a cause of mortality overall in the Western World, cancer is second only to cardiovascular disease. In particular, cancer affects epithelial tissues and over 90% of tumours are derived from this tissue. This is not surprising since many of the known cancer-causing agents (*carcinogens*) are from natural radiation, in the air we breath and from the foodstuffs we ingest, and epithelial cells are the first line of defence to the outside world in the skin, lungs and gastrointestinal tract. Although a great deal of effort has been devoted to defining the optimum protocols for eradicating each type of *primary tumour*, there are often two confounding factors conspiring against a successful outcome: metastatic spread and the unwanted toxicity of the treatment to normal tissue. The most important cause of mortality is spread of the original tumour to a distant site, a process called *metastasis*. If the site of that spread is to a vital organ, e.g. the liver, then death is likely to be significantly hastened.

As metastases have often developed *before* diagnosis, it is widely believed that the greatest single advancement in cancer management (thus improving prognosis) would be gained through more effective methods of early detection – i.e. an *efficient cancer screening programme*. This is not as simple as it sounds, apart from being able to detect a reliable marker of an asymptomatic tumour mass, the test needs to be relatively non-invasive to ensure high patient compliance and, in these so-called recessionary times, seen to be cost-effective. A number of screening programmes are

already in operation, with cervical screening and mammography probably being the most widely adopted; so-called cost-effectiveness can be improved by actively encouraging participation of the group thought to be at the greatest risk of a particular disease, e.g. mammography for women in the 50–64 age group. Colorectal cancer seems an ideal disease for screening because there is a recognized preinvasive state (the adenoma), and the prognosis for invasive lesions classified as Dukes' A stage (see Fig. 6.6) is good, whereas the more invasive stages have a poor prognosis. However, the screening methods of sigmoidoscopy or faecal occult blood tests do not exactly encourage patient compliance. Likewise, there is poor compliance from workers, particularly those retired, in the rubber and dye industries who are at greatest risk from bladder cancer, where six-monthly urine samples to detect haematuria (blood in the urine caused by the haemorrhage of exophytic growths) could be of use. For many types of cancer it could be argued that the most spectacular reductions in incidence could be achieved through *simply* changing lifestyle – e.g. encouraging people to stop smoking and reduce their intake of dietary fat!

The greatest obstacle to the effective eradication of disseminated malignant disease by cytotoxic drugs is the inflicted life-threatening toxicity to normal tissues. The drugs currently in use are perfectly good at killing proliferative cells in tumours, but unfortunately they also kill proliferative cells elsewhere, e.g. in the bone marrow, gut and hair follicles. Thus the treatment of the tumour is limited by the amount of damage which can be tolerated by the normal tissues, particularly the bone marrow, and suitable treatment-free intervals must be scheduled to allow for recovery of the normal tissue. In the case of the bone marrow these intervals may be shortened by courses of recombinant growth factors to correct the induced myelosuppression. The rapid advancements in knowledge of the molecular mechanisms underlying the neoplastic process certainly promise radically new approaches to cancer treatment (see Chapter 7, section 7.4), superseding the relatively crude sledgehammer approaches currently adopted.

Oncology is that branch of medicine that deals with *tumours*, and is literally the science of new growths (Gr. *ogkos*, a swelling; *logos*, science). A tumour (L. *tumor*, swelling) is a swelling caused by excessive continued growth of cells in a tissue, which may be *benign* or *malignant*, while the term *neoplasm* means a new and diseased form of tissue growth. In clinical practice, the words 'neoplasm' and 'tumour' are often used interchangeably. However, the term *cancer* is loosely used to mean any malignant growth, being derived from the Latin meaning 'crab', since the general outline of many malignant growths resembles a crab with the body being the main tumour mass and the claws being the invasive tumour margins. Tumours have been defined as 'masses of tissue whose growth exceeds and is uncoordinated with that of normal tissues, and which persist in the same excessive manner after cessation of the stimuli that evoked the change' (R.A. Willis, *Pathology of Tumours*, 1948). This latter point highlights an important distinction between neoplasia and hyperplasia; neoplasia persists after the removal of the stimulus because of heritable genetic defects in the affected cells, but the elevated cell proliferation rate seen in hyperplastic tissue *ceases* after removal of the stimulus that evoked the change, e.g. after skin wounding the

excessive epidermal proliferation ceases once the tissue defect is healed. Of course, cell proliferation is a normal property of all tissues during embryological development, and it continues throughout adult life in some of them, e.g. bone marrow, gut and skin. These tissues and the glandular epithelia (e.g. liver, kidney and adrenal) can all become hyperplastic to effect wound healing, while in the immune system there is continuous selection of newly derived T- and B-cell clones. All these proliferative reactions are precisely controlled, but it is worth remembering that tumour growth *is not uncontrolled*, for if it were any tumour might soon overwhelm its host in size. Of course, Willis' definition is not by any means complete since excessive growth is not the only behavioural abnormality exhibited by the affected cells: local invasion and the capacity for colonization at distant sites being the most obvious. Furthermore, tumour cell populations are not totally anarchic. They often exhibit patterns of differentiation which to a greater or lesser degree resemble their tissue of origin. In summary, all facets of tumour behaviour, *viz.* cell proliferation, cell death, differentiation, invasion and metastasis appear to be the result of the inappropriate or aberrant expression of probably many genes regulating the cell phenotype.

1.2 Identifying tumours

Imaging techniques

The presence of a tumour at or near the surface of the body provides no problem of accessibility to the clinician, and, for example, testicular and prostate cancer and even lymph node metastases are initially investigated by palpation. Other tumours such as those of the bladder, cervix and rectum are often diagnosed by the clinician by endoscopy before referral to the Radiology Department. However, tumours within the body mass are better revealed by more sophisticated imaging methods. The most widely available investigative procedure is *diagnostic radiography*. The simple radiograph or the use of X-irradiation after the administration of radio-opaque dyes provides information of the extent of local growth of a tumour (Fig. 1.1A, B). Conventional radiology provides the highest spatial resolution, and is therefore best suited to the gastrointestinal tract and bone where fine detail can be shown.

X-rays are produced whenever high speed electrons are brought abruptly to a halt, commonly by a block of tungsten. A high voltage is applied so that the electrons are attracted to the 'target' (positive electrode), and upon striking the target the acquired kinetic energy is surrendered and converted into other forms, one being X-irradiation. In diagnostic radiology, a beam of X-rays is directed at the patient, some are stopped (absorbed), some are deflected (scattered) and some pass through unaffected (transmitted). The X-ray beam emerging from the patient is the result of these events, but the human eye is not visually sensitive to X-irradiation. However, photographic film exposed to X-rays and then developed will be found to be blackened – the irradiation has affected the emulsion of silver salts so that after development, metallic silver is released and the film or paper appears dark. The amount of silver released obviously depends on the level of radiation to which the film is subjected, 'the expo-

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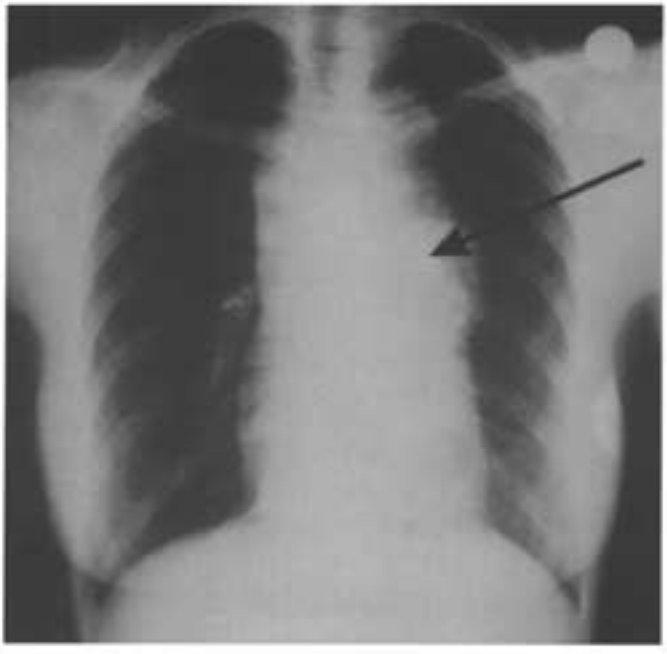


(B)

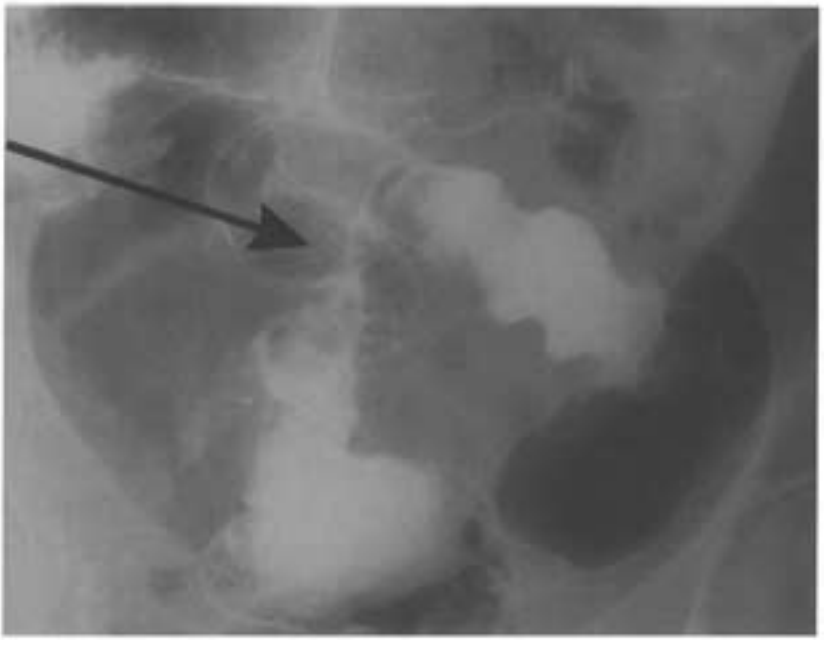


Fig. 1.1 (A) Plain chest radiograph of a patient with tumour involvement of mediastinal lymph nodes as indicated by increased opacity (arrow) and (B) a tumour of the large intestine indicated by an irregular defect (arrow) in the lumen which is highlighted by an opaque contrast medium.

sure', thus regions of the film exposed to a lot of X-rays are black, while regions exposed to few X-rays appear relatively light – hence bones which are very dense, absorb X-rays and are outlined as light-coloured structures (Fig. 1.1A). The final film is therefore in the form of a negative, dark where there is little X-ray shadow, and bright where the film has been shaded by dense tissue, e.g. bone.

A three-dimensional image of the subject can be formed with the aid of *computed tomography* (CT), in which the information from numerous small X-ray beams is digitally integrated. CT has in fact been one of the most spectacular advances in medicine over the last few years, and since the installation of the first prototype in 1972 the technology has swiftly advanced. X-ray CT scanning relies on the principle of reconstruction from projections, and a three-dimensional (3-D) image of the patient in cross-section can be obtained (Fig. 1.2); 3-D images may be thought of as a set of stacked 2-D tomograms. Basically the X-ray tube rotates around the patient, and the images are collected either by detectors which rotate synchronously with the X-ray tube round the patient through 360°, or by a complete ring of stationary detectors (Fig. 1.3). CT has a higher tissue density discrimination than conventional radiology and is, therefore, useful for the diagnosis of tumours of the abdominal organs and

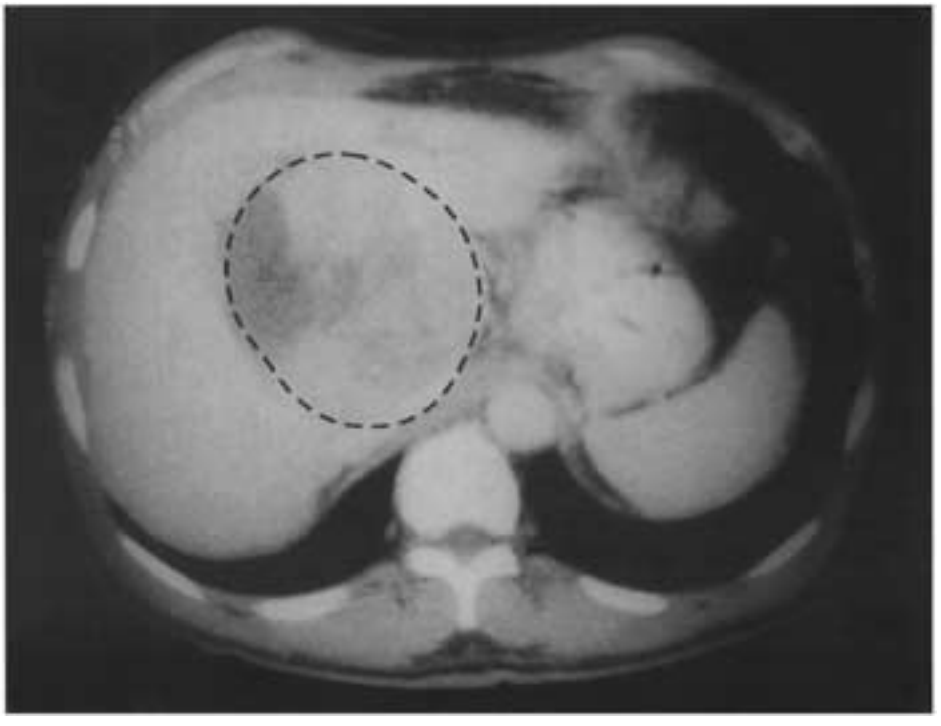


Fig. 1.2 CT scan through the abdomen showing the presence of a hepatocellular carcinoma as an area of diminished density (encircled), here more X-rays have been transmitted through this area and the film is more blackened. (Image kindly supplied by Dr. Pat Price, Department of Clinical Oncology, Hammersmith Hospital.)

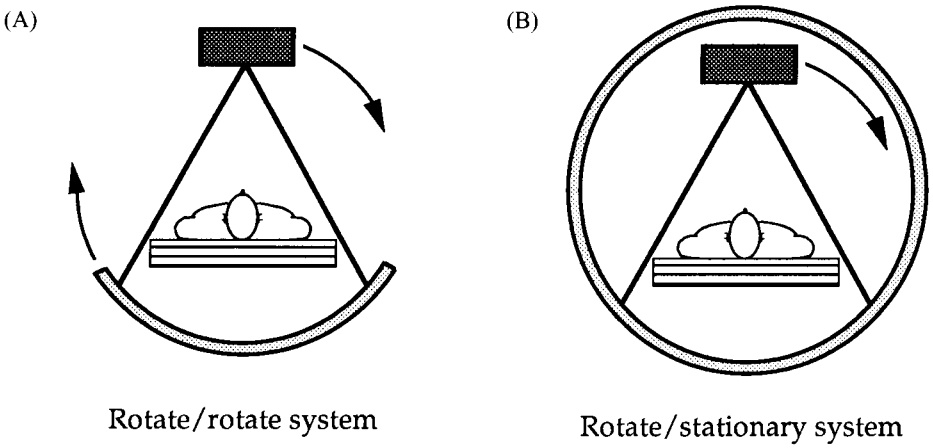


Fig. 1.3 Schematic diagram illustrating the two basic types of CT scanner: (A) either the X-ray tube and the detectors rotate synchronously round the patient through 360° – the *rotate/rotate* system, or (B) there is a complete ring of stationary detectors and the X-ray tube rotates around the patient within the ring – the *rotate/stationary* system.

brain (see Figs. 1.2 and 1.4) where small differences in density between soft tissue, fluid, fat and other structures can be discerned.

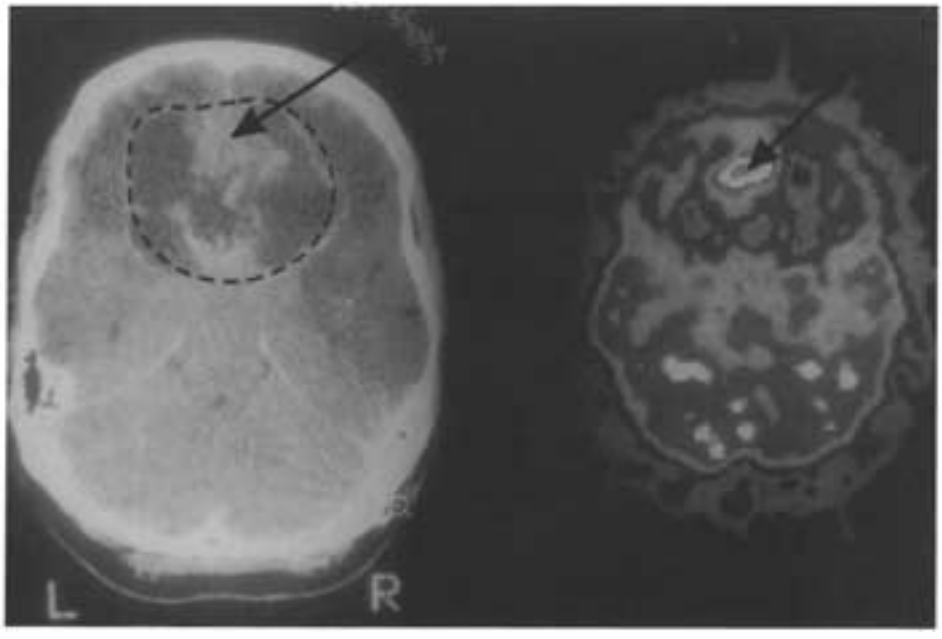
Magnetic resonance imaging (MRI) is another technique for producing high-resolution fully 3-D tomographic data sets, and therefore like CT, is useful for planning radiotherapy geometrically tailored to the target – *conformal therapy*. Like X-ray CT, MRI is highly suitable for displaying soft tissue detail. The method employs radiofrequency radiation in the presence of carefully controlled magnetic fields, and basically portrays the distribution of hydrogen nuclei and parameters relating to their motion in water and lipids. Any nuclei with a net charge are suitable for MRI, but most data have been on the nuclear magnetism of the hydrogen nucleus (or proton) because of its ubiquitous distribution in biological material.

Nuclear medicine techniques assess organ and tissue function by observing the distribution of an injected radiopharmaceutical. It is hoped that the kinetics and/or distribution of the pharmaceutical are different between the normal and tumour tissue, and this can be detected by localizing the radioactivity emitted from the radiopharmaceutical. This radioactivity can be imaged by a gamma-camera, a process called *scintigraphy*. Obviously a pharmaceutical with appropriate biological behaviour must be chosen, the bound radionuclide must not affect the biological behaviour, while the half-life of the radionuclide should be sufficiently long to complete the imaging process without presenting the patient with an unnecessary radiation burden. So, for example, technetium-99m (^{99m}Tc) is widely used for scintigraphic studies because of its short half-life (six hours), and in the search for skeletal metastases ^{99m}Tc-diphosphonate is used since bony metastases are associated with increased blood flow and osteoblastic (bone-forming) activity – hence increased radiopharmaceutical uptake (Fig. 1.5).

Positron emission tomography (PET) is a nuclear medicine procedure that is a form

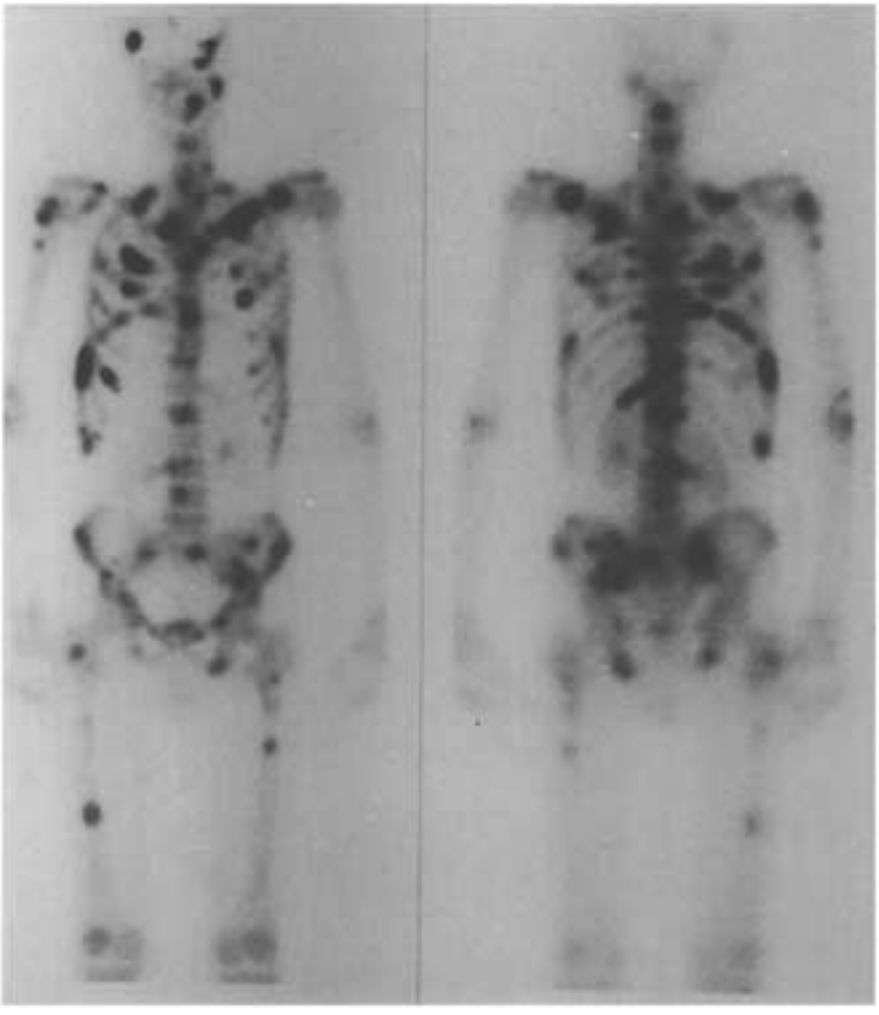
of sectional imaging of a radiopharmaceutical in which the radionuclide is a positron emitter, and is based on the coincidence detection of high energy photons from positron emission. Unlike CT and MRI, which provide images of precise anatomical localization, PET aims to provide information on metabolic differences between normal and neoplastic tissue (see Fig. 1.4). A positron emitted during radionuclide decay is very rapidly 'captured' by an electron, and results in a burst of radiation in the form of two 511 KeV photons travelling at 180° to each other. By encircling the patient with a ring of radiation detectors, it is possible to record these pairs of photons emerging from the body in opposite directions, using coincidence circuitry which thus defines the line along which the decay must have occurred (Fig. 1.6). If enough coincidental counts are made then a complete tomographical representation of the distribution of radioactivity can be obtained by computer reconstruction in much the same way as in CT.

Ultrasonography relies on the differing echo patterns which tissues create when bombarded by sound from an ultrasonic generator. Echoes are generated at interfaces of tissues whose density differs, but they cannot be obtained if the organ is shielded by bone, since bone reflects all the sound from the beam. Additionally, ultrasound is unable to cross a tissue–gas boundary, so bowel gas is another barrier to adequate



(A) (B)

Fig. 1.4 Imaging of a brain tumour by CT scan (A) and the same tumour imaged by positron emission tomography (PET) (B) after administration of ^{18}F-labelled 2-fluoro-2-deoxyglucose (^{18}F-FDG). The CT scan shows a cystic tumour in the frontal lobe (encircled) with a solid component anteriorly (arrow) which corresponds to the hot spot in the PET ^{18}F-FDG scan (arrow) indicating an area of high glucose uptake – a recognized marker of neoplastic growth. (Images kindly supplied by Dr. Pat Price, Department of Clinical Oncology, Hammersmith Hospital.)



(A) (B)

Fig. 1.5 Whole-body bone scintigraphy, four hours after injection with 550 MBq of ^{99m}Tc-diphosphonate. Focal increased activity in all areas of the skeleton is compatible with multiple skeletal metastases. (A) Shows the anterior view and (B) shows the posterior view. (Kindly supplied by Daphne Glass, Radiology Department, Hammersmith Hospital.)

visualization. Ultrasound is an excellent technique for imaging in the liver, and metastases down to 1 cm diameter can be reliably detected (Fig. 1.7).

Histological techniques

The investigations mentioned above reveal the space occupying properties of a tumour, which might provide an indication of *stage* (see Chapter 6, section 6.3) of tumour development, however, the *histogenesis* and *grade* (see below and Chapter 6, section 6.2) of the tumour are determined after surgical removal and microscopical

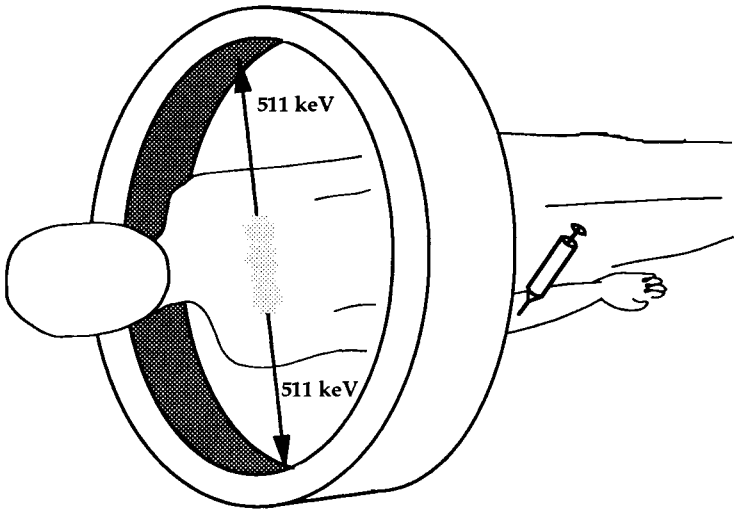


Fig. 1.6 Schematic representation of the positron emission tomography (PET) scanner, showing the recording, by coincidence circuitry, of pairs of photons of similar energy emerging from the body in opposite directions.

assessment. This morphological diagnosis takes the form of an examination of either cells (*cytological* diagnosis) or of tissue sections (*histopathological* diagnosis). In practical terms, clinical cytology is divided into two types (*exfoliative* and *aspiration*) depending upon the means used to collect the material. Exfoliative cytology comprises all examinations carried out on cells which are normally desquamated from surfaces or dislodged from them by mechanical means such as by spatulae or brushes, while aspiration cytology is the examination of cells collected by a thin needle attached to a syringe. Exfoliative cytology can be used in the diagnosis of tumours of the respiratory and urinary tracts by simply looking at sputum and urine samples, though the exact location of the tumour cannot be identified. Probably the most important use of exfoliative cytology is in the mass screening programme to detect cervical intraepithelial neoplasia (Fig. 1.8A, B), a well recognized preinvasive lesion (see Chapter 4, section 4.3 and Fig. 4.2). Fine needle aspiration cytology can be used in the diagnosis of palpable suspect lesions in tissues such as breast, prostate and lymph node, and with the advent of accurate imaging techniques it can also be applied to the examination of non-palpable deep-seated lesions in all parts of the body (Fig. 1.9).

When tissue is used for histopathological diagnosis, a biopsy may be obtained by a variety of means. In the respiratory, gastrointestinal and genitourinary tracts a variety of fibre optic-guided endoscopy instruments can be used for sampling, while often the diagnosis is made after complete resection of the tumour (excision biopsy). As discussed above, to observe tissues at the cellular level one has to use a microscope, and for the commonest modes of observation including bright field microscopy and transmission electron microscopy, the illumination needs to pass through the tissue, thus requiring the tissue to be in the form of thin sections. Clearly, cutting sections

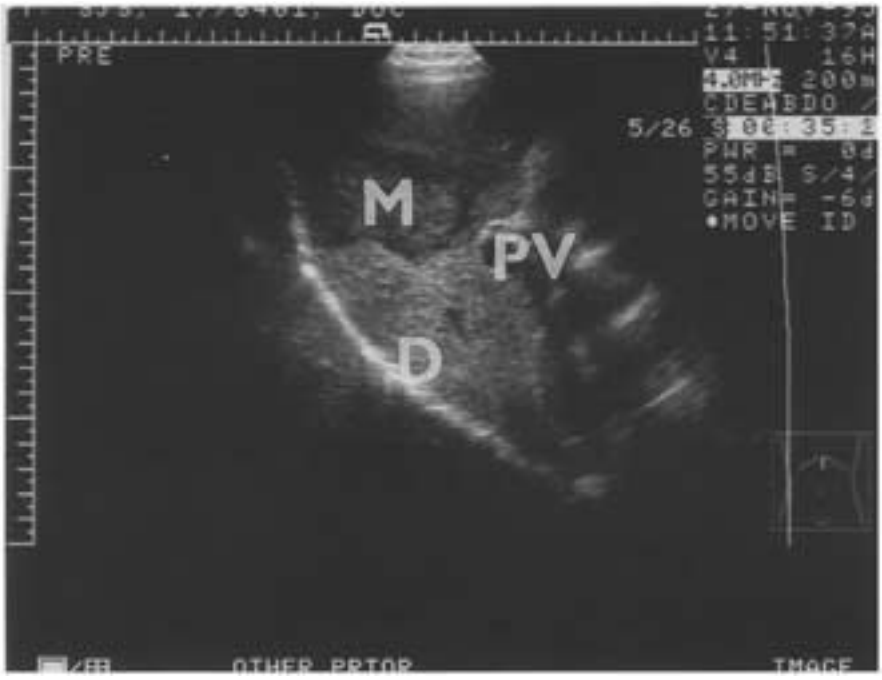


Fig. 1.7 A typical pie-shaped field of view obtained by a sector plan ultrasound sweep. Here a large liver metastasis (M) from an oesophageal carcinoma is seen as a heterogeneous lesion of generally lower reflectivity than the surrounding liver. Conventionally the strength of echoes is represented by degrees of brightness in a grey scale image, the brighter the image the stronger the reflection. This is a longitudinal section through the liver as depicted by the plane of section (small vertical line) in the body icon in the bottom right. D, diaphragm; PV, portal vein. (Kindly supplied by Dr David Cosgrove, Department of Diagnostic Radiology, Hammersmith Hospital.)

of tissue thin enough to be transparent is not possible without prior preparation of the tissue. These considerations are served either by *fixing* then *embedding* the tissue in a rigid medium, or by *rapid freezing* followed by cutting frozen sections. Fixation is the term used to describe chemical preservation of tissue, by cross-linking and/or precipitation of proteins, so that the architecture it had while viable is retained. Samples, therefore, need to be fixed immediately they are removed to preserve the tissue in its original form; this requires killing of bacteria and moulds, inhibition of the activity of autolytic enzymes and prevention of decomposition, as well as maintainance of osmotic differentials. Tissue also needs to be given a texture which permits easy sectioning – not brittle, not soft, and it must be robust enough to avoid damage in subsequent processing. Sectioned tissue is finally counterstained for observation, so the fixation methods need to render the tissue receptive to the proposed stains. All fixatives cause tissue shrinkage to a greater or lesser extent, and with the removal of water in later stages of processing there can be as much as a 50% reduction in volume; this needs to be borne in mind when measurements are made on processed tissue.

Several fixative mixtures are based on formaldehyde, which is a colourless gas, very

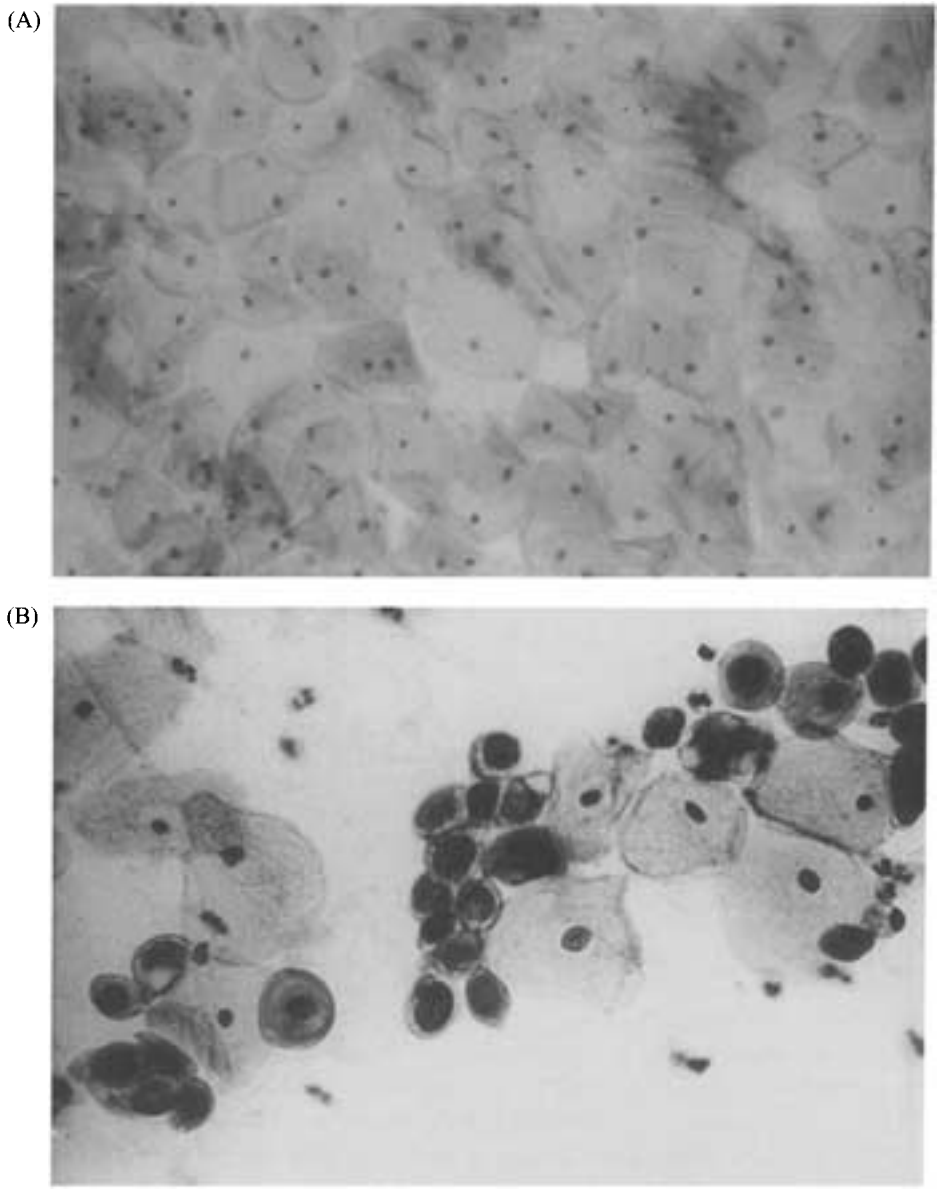


Fig. 1.8 (A) Superficial squamous (scale-like) cells from a cervical smear of normal uterine cervix having typical pyknotic (shrunken) nuclei and a very low nuclear:cytoplasmic ratio. By contrast (B) in a case of cervical intraepithelial neoplasia (see also Fig. 4.2) many of the cells have not differentiated into squamous cells, but have remained small with a high nuclear: cytoplasmic ratio, often with irregular nuclear shapes (nuclear pleomorphism) – features of neoplastic cells. (See Chapter 4, section 4.7 and Fig. 4.18.)

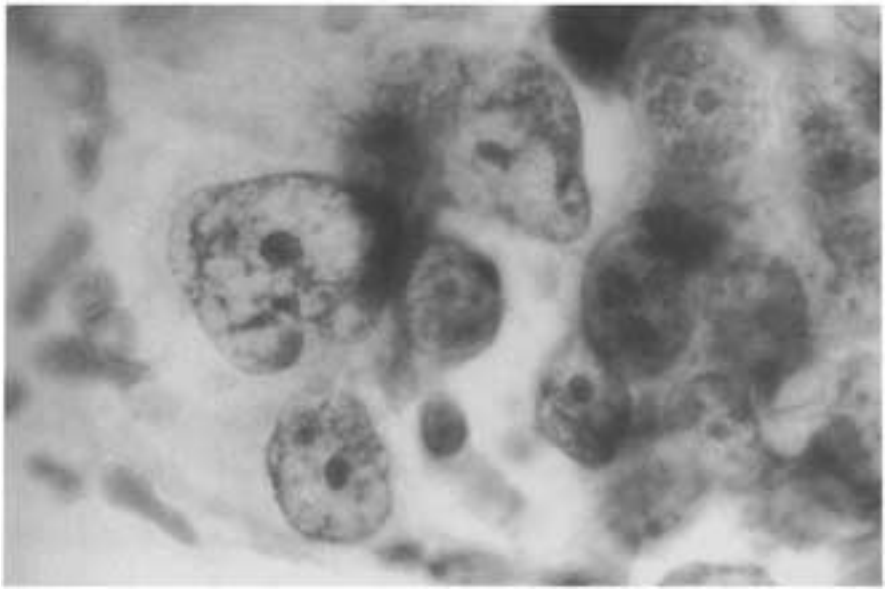


Fig. 1.9 A fine needle aspiration cytology specimen of lung tissue, illustrating the pleomorphic nuclei of a large cell anaplastic lung carcinoma. (Kindly supplied by Ian Phillips, Cytology Department, Hammersmith Hospital.)

soluble in water and generally used as a 4% aqueous solution. The word 'formalin' is used for the commercial saturated solution of formaldehyde in water which is approximately 40% weight/volume (w/v); the working solution is thus 10% formalin. Neutral buffered formalin is the most commonly used fixative in pathology laboratories because of its wide-range applicability and low cost. Tissue from archives very many years old can always be referred back to, and because formalin is cheap, reference material as big as whole organs (or bodies) can be preserved for museums. The chemical properties of neutral buffered formalin mean that treated tissue is suitable for dissection even after fixation and it is the only fixative that will accept many of the silver impregnation stains so useful, for example, for neurones and their processes.

Except for frozen samples (see below), fixed tissue needs to be embedded in a rigid block to render it amenable to sectioning. For light microscopy the most common medium is paraffin wax, although some synthetic resins, such as the methacrylates, are commonly employed. Whichever medium is to form the block, the tissue has to be *dehydrated* to accept impregnation by the liquid embedding medium, which then solidifies into its final form. Tissue sections are cut on a microtome and the sections are collected on glass slides – wax sections usually have a thickness of 5 μm.

In certain circumstances instead of fixing and embedding tissue, preservation and sectioning can be achieved by freezing tissue blocks. This is the method of choice for very rapid assessment of biopsies during surgery, and also for the preservation of labile antigens. Frozen sections are cut on a cryotome, essentially a microtome permanently mounted inside a freezing cabinet. Although excellent preservation can be

obtained with care, frozen sections are generally of poorer quality than those from fixed and embedded tissue.

To examine the tissue microscopically it has to be stained. Tissue staining is performed in stages, first involving removal of wax from the section on the glass slide by an appropriate solvent. This is necessary because wax is immiscible with aqueous solutions. Then water is introduced gently into the sections through a series of graded alcohols, without this, tissue architecture would become distorted. The process of tissue rehydration is generally referred to as 'taking down to water'. The routine examination of sections is normally accomplished after staining with the combination of *haematoxylin* and *eosin* dyes (H&E).

Haematoxylin is a cationic dye, behaving as positively charged dye ions and reacting with negatively charged tissue groups, imparting a blue/purple colouration to the 'basophilic' components. Nucleic acids, both DNA and RNA, are the principal basophilic cell constituents, hence nuclei and ribosome-rich areas are prominently stained (Fig. 1.10 A, B, C). Eosin, however, is an anionic dye acting as negatively charged dye ions which react with positively charged tissue constituents. 'Acidophilic' components stain varying shades of orange to red, and include cytoplasmic proteins, abundant extracellular proteins like collagen, the haemoglobin in red blood cells and mitochondria; hence the strong staining of muscle cells (Fig. 1.10B).

Though much of diagnostic histopathology requires only H&E stained tissue sections, the science of *immunocytochemistry*, developed over the past 50 years, has revolutionized the field. Immunocytochemistry is the use of labelled antibodies as specific reagents for the localization of tissue constituents, and its application has removed much of the uncertainty from diagnosis which relied on special stains and 'educated guesswork'; antigen–antibody reactions are absolutely specific so positive identification of tissue constituents can be achieved. Of course, like any technique, false negatives and false positives through inadequate precautions must be guarded against.

Immunocytochemistry plays a vital role in diagnostic tumour pathology in circumstances where morphology alone cannot reliably be used to infer the tissue of origin, or, for example, where the identity of a secreted hormone is unknown. In addition, immunocytochemistry plays a vital role in understanding the biology of neoplastic growth, and the technique has been applied to examining all facets of cell behaviour: the expression of transcription factors, growth factors and their receptors, cell adhesion molecules and measurement of proliferation.

The use of immunocytochemistry enables the localization of the antigen of interest in cytological material as well as in histological sections at both light and electron microscope levels. Antigens are usually demonstrated by an *indirect technique* (Fig. 1.11), the simplest of which involves the use of a *primary antibody* to detect the antigen of interest, and then a labelled *secondary antibody* to detect the primary. The label conjugated to the secondary antibody can be a fluorescent marker such as fluorescein isothiocyanate, which can then be visualized by fluorescence microscopy. More commonly, the label is an enzyme, usually horseradish peroxidase, which is demonstrable by its ability to reduce H_2O_2 to water in the presence of an electron donor.

The electron donor is commonly diaminobenzidine, which is oxidized to a coloured final reaction product which is an insoluble precipitate that survives long-term storage (Fig. 1.12).

Many modifications to these relatively simple procedures have evolved in order to improve the sensitivity of the immunostaining, i.e. bind more marker enzyme to each primary antigen site. Avidin/biotin techniques are the most sensitive methods of this genre. These substances are both naturally occurring components of egg which have a great affinity for each other, with avidin, a large basic glycoprotein from egg white, having four high affinity biotin binding sites, biotin being a vitamin found in egg yolk,

(A)

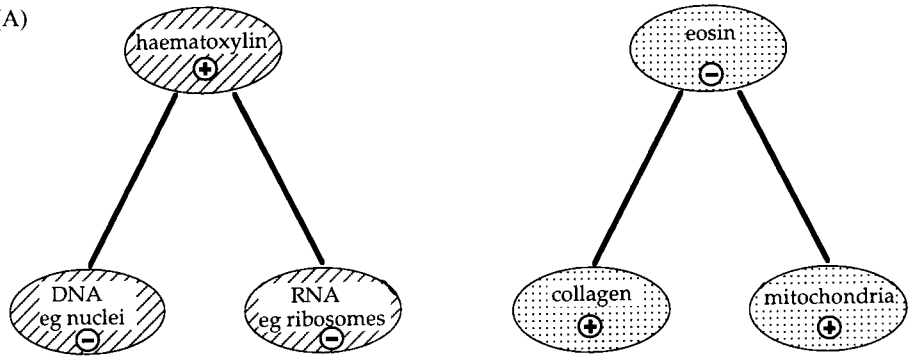
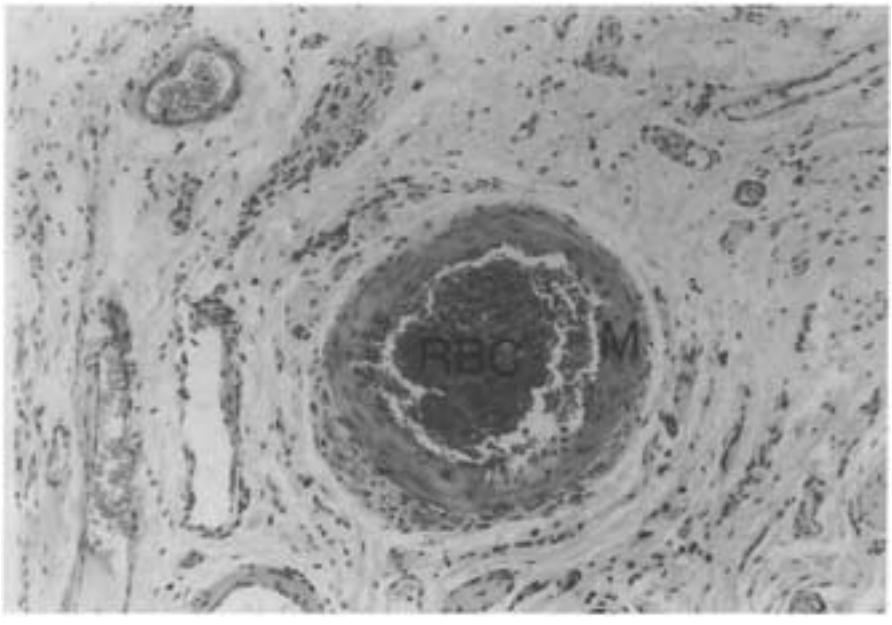


Fig. 1.10 (A) The principle of haematoxylin and eosin staining resulting from electrostatic binding. (B) Nucleic acids are basophilic, staining blue/purple (dark staining) as seen in nuclei, whereas muscle cells (M) and red blood cells (RBC) are intensely eosinophilic staining orange/red. (C) *opposite* The RNA-rich perinuclear cytoplasm (arrows) of neurones is also intensely basophilic.

(B)

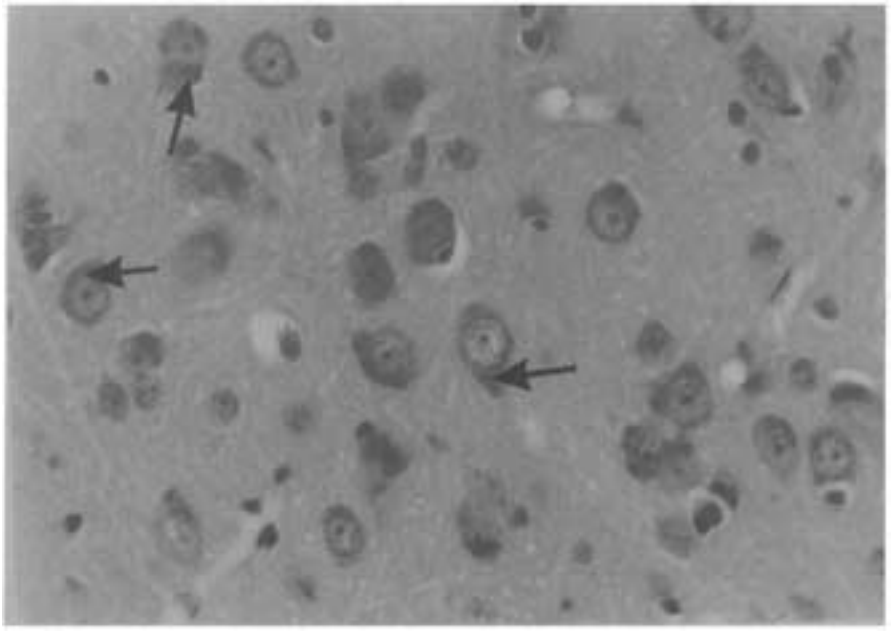


thus adding greater sensitivity over simple indirect methods. Both can be conjugated to antibodies, peroxidase or fluorescent markers but the most common technique is that of the ABC technique (*a*vidin/*b*iotin *c*omplex) in which the secondary antibody is labelled with biotin and this is recognized by an avidin/peroxidase complex final layer (Fig. 1.13). Streptavidin, a 60 kDa protein (also with four biotin-binding sites) from the bacterium *Streptomyces avidinii* is often used in place of avidin. Streptavidin reduces background staining because its near neutral isoelectric point eliminates electrostatic binding, and in addition, its absence of carbohydrate side-chains ensures it does not bind to tissue lectins.

At electron microscope level simple indirect techniques are generally used. The primary antibody is applied and this is followed by either a peroxidase conjugate (which is then rendered electron dense by osmication) or a colloidal gold conjugated secondary antibody. Colloidal gold is available in a variety of particle sizes from 1 nm to 40 nm, the most convenient being in the 10–20 nm range. Colloidal gold is preferred to peroxidase when the antigen of interest is localized to already electron-dense structures such as the endocrine granules of endocrine cell tumours (Fig. 1.14).

Lectins are proteins or glycoproteins frequently of plant origin, used to localize carbohydrates; in nature they may be concerned with the recognition of host plant roots by symbiotic bacteria. Specificity and binding of lectins is not an immune process, but because each lectin specifically recognizes a certain carbohydrate moiety they can be used as accurate markers. A lectin in common use is *Ulex europaeus* agglutinin (UEA 1), which recognizes α-L-fructose and binds to terminal fucosyl groups linked to certain oligosaccharides. It agglutinates erythrocytes of blood group

(C)



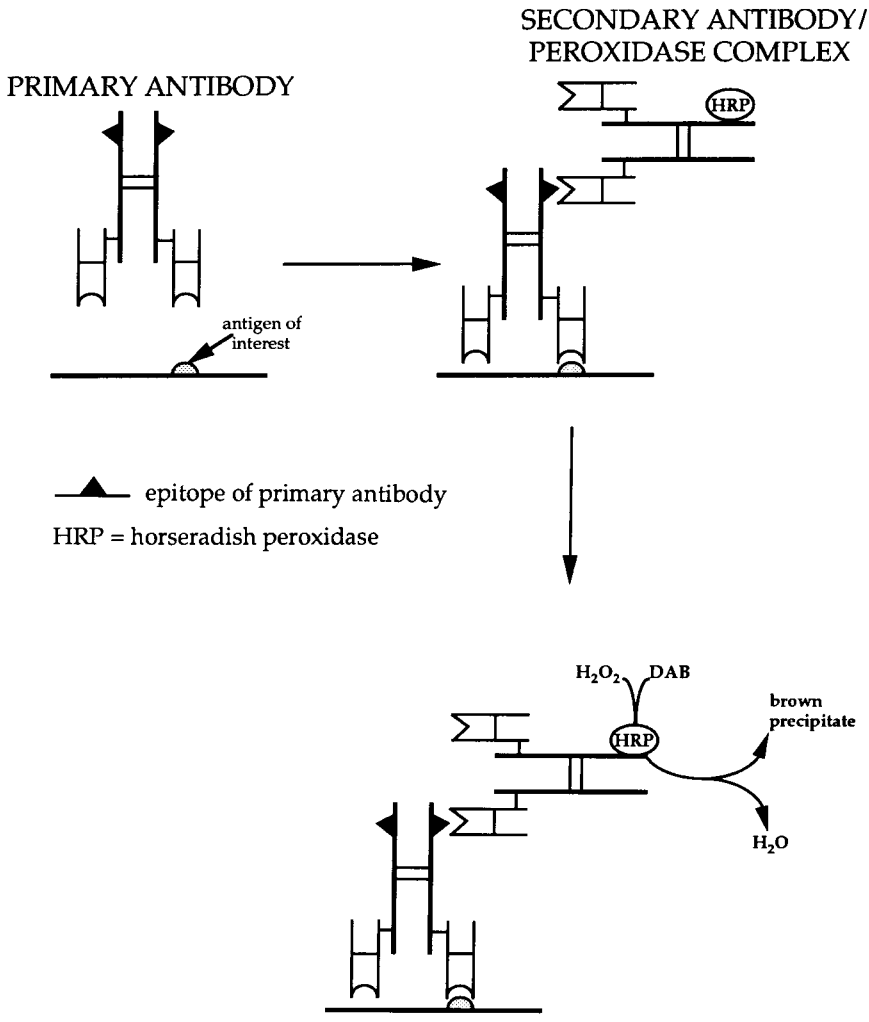


Fig. 1.11 The principle of immunocytochemistry using a simple indirect technique, with the secondary antibody conjugated to horseradish peroxidase (HRP). HRP has the ability to reduce hydrogen peroxide to water in the presence of an electron donor such as diaminobenzidine (DAB), forming a brown-coloured final reaction product.

O as they contain α-fucosyl groups, and similarly has an affinity for such carbohydrates associated with endothelial cells and some tumours, particularly those associated with the vascular endothelium such as angiosarcomas. Lectins are multivalent, so in addition to the carbohydrate they identify, they can be bound to a label such as biotin which can then be visualized by an avidin–biotin–peroxidase complex.

The demonstration of the presence of a polypeptide antigen in a tissue or cell by immunocytochemistry imparts no definitive information about the processes that have lead to its synthesis. Hybridization is the technique by which specific sequences of DNA and RNA can be identified by matching them up to complementary labelled 'probes'. In fact